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Field studies on infectious reproductive diseases and lameness  
in sows

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ACADEMIC DISSERTATION

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# 1. ABSTRACT

More than half of the sows in Finland are culled every year. The most common reason for culling is fertility problems, followed by lameness. In the absence of other infectious reproductive diseases, the main diseases to consider are porcine parvovirus (PPV) and vulvar discharge syndrome (VDS). The basic epidemiology, including prevalence in Finland and associative factors of PPV, VDS and lameness, has not previously been studied.

The objectives of this study were to investigate PPV, VDS and lameness among loosely housed sows by determining their prevalence and effect on fertility. In addition, the response of gilts to PPV vaccination was followed with haemagglutinin inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) tests after PPV vaccination and examined with regard to reproductive success. Bacterial growth, antimicrobial susceptibility and cytological and vaginoscopic findings were evaluated in VDS animals, and the potential usefulness of two acute-phase proteins, haptoglobin (Hp) and C-reactive protein (CRP), in VDS and in lameness diagnostics was assessed.

To examine the herd prevalence of high PPV titres, a minimum of 11 blood samples on each of 21 randomly chosen farms were drawn. Samples were analysed with HI test. High PPV titres was found to be common; on 17 farms (81%) at least one animal had a high titre ( $>1:512$ ), and 44% of all animals sampled had a high titre. The vaccination programmes had many shortcomings. The factors found to have a significant effect on HI titres were herd size and parity of two or higher. Non-return rate, rebreeding interval and litter size did not differ between herds with no high HI titres and those with at least one high HI titre.

Thirty-nine gilts from four herds endemically infected with PPV were injected twice with a commercial PPV vaccine. The antibody response was studied, revealing a consistent increase in humoral immunity. PPV antibodies were analysed both with HI and ELISA, and the agreement between them was moderate. A potential association between high antibody titres and reproductive failure (repeat breeding, culling for infertility,  $\leq 6$  piglets born alive) was also investigated, but no association was observed.

The effect of VDS on sow and gilt fertility was evaluated on 26 farms. Fewer VDS animals (47.4%) than non-VDS animals (68.3%) farrowed at the first chance after the examination. Environmental and individual factors likely to be associated with fertility and VDS were tested. Factors associated with farrowing were VDS, reproductive status, availability of roughage and confinement to individual stalls. None of the variables tested was associated with VDS. There was an absence of a systemic acute-phase response, as indicated by low concentrations of Hp and CRP.

A total of 21 sow herds were randomly chosen to determine the prevalence of VDS. The median VDS prevalence was low, 0% (range 0-4.5%). Nine of the 655 animals (1.4%) examined displayed signs of VDS. Detection of vulvar discharge was associated with vaginoscopic examination findings and with bacteriology, but the association with leukocyte counts was weak. Antimicrobial susceptibility testing was performed on pure bacterial cultures. Seven of the 16 isolates (44%) were considered to be resistant or to have intermediate susceptibility to at least one of the antimicrobial agents (sulpha-trimethoprim and ampicillin) tested.

Lameness was a frequent finding in the 21 herds studied: 8.8% of the animals were lame. Lameness had higher Hp and CRP concentrations than sound-footed controls. Animals housed on slatted floors had 2-fold odds of being lame and 3.7-fold odds of being severely lame as compared with animals housed on solid floors. Higher parity and use of roughage increased the odds of farrowing; however, lameness did not increase the risk for not farrowing.

In conclusion, high PPV titres and lameness were common and VDS rare in a Finnish loose-housed sow population. VDS impaired fertility, but lameness did not. Lameness had higher Hp and CRP levels than controls, but VDS animals did not react with APPs. Animals housed on slatted floors had higher odds of being lame than animals on solid floors.

## 2. LIST OF ORIGINAL PAPERS

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals.

- I) Oravainen J, Heinonen M, Tast A, Virolainen J, Peltoniemi OAT, 2005: High porcine parvovirus antibodies in sow herds: prevalence and associated factors. *Reprod Dom Anim* **40**, 57–61.
- II) Oravainen J, Hakala M, Rautiainen E, Veijalainen P, Heinonen M, Tast A, Virolainen JV, Peltoniemi OAT, 2006: Parvovirus antibodies in vaccinated gilts in field conditions – results with HI and ELISA tests. *Reprod Dom Anim* **41**, 91–93.
- III) Oravainen J, Heinonen M, Seppä-Lassila L, Orro T, Tast A, Virolainen JV, Peltoniemi OAT, 2006: Factors affecting fertility in loosely housed sows and gilts: vulvar discharge syndrome, environment and acute-phase proteins. *Reprod Dom Anim* **41**, 549–554.
- IV) Oravainen J, Heinonen M, Tast A, Virolainen JV, Peltoniemi OAT, 2008: Vulvar discharge syndrome in loosely housed Finnish pigs: prevalence and evaluation of vaginoscopy, bacteriology and cytology. *Reprod Dom Anim*, doi: 10.1111/j.1439-0531.2007.00852.x.
- V) Heinonen M, Oravainen J, Orro T, Seppä-Lassila L, Ala-Kurikka E, Virolainen JV, Tast A, Peltoniemi OAT, 2006: Lameness and fertility of sows and gilts in loose-housed herds. *Vet Rec* **159**, 383–387.

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### 3. ABBREVIATIONS

Ab	antibody
APP	acute-phase protein
APR	acute-phase response
b	coefficient
BCS	body condition score
CI	confidence interval
CRP	C-reactive protein
ELISA	enzyme-linked immunosorbent assay
Evira	Elintarviketurvallisuusvirasto (Finnish Food Safety Authority)
G	gauge
GEE	generalized estimating equations
HI	haemagglutinin inhibition
Hp	haptoglobin
MMA	mastitis metritis agalactia
OC	osteocondrosis
OR	odds ratio
P	P-value
PPV	porcine parvovirus
PPV-NS1	porcine parvovirus non-structural protein 1
PRRS	porcine reproductive and respiratory syndrome
sd	standard deviation
SE	standard error
SPF	specific pathogen-free
VDS	vulvar discharge syndrome
z	design matrix for random effects

## **4. INTRODUCTION AND REVIEW OF LITERATURE**

### **4.1 GENERAL INTRODUCTION**

In 2005, there were 590 herds, with a mean of 70 sows per herd, in the National Litter Recording Scheme of the Finnish Animal Breeding Association (Anonymous 2005). Altogether 24307 sows were culled or died spontaneously. Thus, 59% of all sows are replaced every year. An American study with 32 commercial herds revealed a similar replacement rate (Rodriguez-Sas et al. 2003), while a Swedish study with data from 21 piglet-producing herds showed a removal rate of 49.5% (Engblom et al. 2007). The main cause was infertility problems (29%), followed by leg problems (17%). In 6% of cases, the cause was a planned removal from the herd, and in 8% spontaneous death. In the Swedish study, lameness and/or foot lesions were causes of removal in only 8.6% of the sows (Engblom et al. 2007).

Many of the fertility problems can be avoided by good stockmanship and planning (Svendsen and Steen Svendsen 1997; Arey and Edwards 1998) and high-quality floors (Munsterhjelm et al. 2006). Fighting, which occurs during mixing of animals, results in stress that can impair reproductive performance. However, the detrimental effects on reproduction can be minimized by careful timing of regrouping (Arey and Edwards 1998). Nevertheless, when fertility problems do occur, infections are frequently cited as the cause. Since we do not have brucellosis, pseudorabies or porcine reproductive and respiratory syndrome (PRRS) in Finland (Anonymous 2004), the infectious causes to consider are porcine parvovirus (PPV) and vulvar discharge syndrome (VDS). The situation is somewhat similar to specific pathogen-free herds in other countries.

The basic epidemiology, including prevalence, of PPV, VDS and lameness in Finland has not been studied before. These diseases can cause considerable problems on individual farms despite apparently good management.

A big change is taking place in sow housing throughout Europe. European legislation that prohibits keeping gestating sows individually confined for extended periods of time takes effect in January 2013 (European Union 2001). Changes in housing systems from individual crates to group housing are likely to complicate the disease situation. Loose housing may bring changes to a herd, including bigger herd size, more recruitment animals and younger age structure. This can easily lead to new problems; gilts are more susceptible to certain diseases (Johnson et al. 1976, Dee 1992), and new premises can be a challenge to immunity (Wattrang et al. 1998). More information is needed on the risk factors for PPV, VDS and lameness and the prevalence of these conditions before preventive measures and evaluation of their possible effects on the economy of sow herds can be undertaken.

### **4.2 PORCINE PARVOVIRUS (PPV)**

PPV, established by the demonstration of antibodies in serum samples or viral antigens in foetal tissues, is a ubiquitous disease (van Leengoed et al. 1983). It is a common cause of reproductive failure in swine herds. Clinical manifestations of PPV infection in a

herd include conception failure, delayed return to oestrus, embryonic and foetal death with resorption, mummification, and small litter size, stillborn piglets, neonatal death and abortion (van Leengoed et al. 1983).

Vaccination is an effective means of preventing PPV-induced foetal loss in gilts (Wrathall et al. 1984; Edwards et al. 1986; Sorensen et al. 1988). Decision-tree analysis clearly shows that it is cost-effective to vaccinate all females in a herd (Parke and Burgess 1993; Gardner et al. 1996). For gilts in field conditions, two doses of a vaccine 2-4 weeks apart are recommended to provide a good and long-lasting immune response (Paul and Mengeling 1986).

Haemagglutinin inhibition (HI) test is based on the ability of PPV to agglutinate erythrocytes. This is the most frequently used test for detecting and quantifying humoral antibodies to PPV (Mengeling 1999). The HI test has been considered to be the standard test for PPV serology, but enzyme-linked immunosorbent assay (ELISA) tests are increasingly being used in many laboratories. Agreement between these two tests is therefore of great interest.

Interpretation of antibody titres is difficult, as practically all farms now vaccinate against PPV. However, having many very high HI titres in a herd provides evidence that PPV infection is or has been present at the farm since vaccination does not result in such high levels of antibodies (Pirjo Veijalainen, personal communication). Animals are considered to have low antibody levels when HI titres are  $\leq 1:512$ ; titres  $> 1:512$  are considered high (Neuvonen et al. 1979, Anonymous 2007). To make a definite diagnosis of a PPV epidemic in a herd, other information is needed as well. Virus isolation or detection of antibody in sera or other body fluids of fetuses and stillborn piglets gives evidence of *in utero* infection (Mengeling 1999). Danish researchers (Madsen et al. 1997) have described a non-structural protein, NS1, which may be used to distinguish antibodies against PPV from vaccination and infection in the future.

Even herds with vaccination programmes occasionally suffer from PPV-like symptoms (Peltoniemi 1999). This challenged us to study PPV titres and vaccination routines in sow herds as well as vaccination success among gilts.

#### **4.3 VULVAR DISCHARGE SYNDROME (VDS)**

Endometritis, metritis, cervicitis, vaginitis and urinary tract disease may result in pathological discharge. The micro-organisms underlying these infections are often the same (Dee 1992). Vaginal discharge varies in duration, quantity and appearance according to the underlying disease. Metritis is characterized by a necrotizing, malodorous discharge with autolysed pieces of foetal membranes and proteinaceous fluid. In endometritis, purulent discharge of varying amounts occurs within 7 days before oestrus and is repeated at 21-day intervals. In cystitis, affected sows' urine typically contains various amounts of pus or blood; discharge is unrelated to oestrus and has an irregular discharge interval. In vaginitis, the discharge interval is irregular and unrelated to oestrus and only a small amount of discharge is present. (Meredith 1991; Dee 1992).

Infections of the urogenital tract are frequently environmental in origin. The housing system has been shown to have an impact on the onset of these infections (Dee 1992).

In most cases, the microbes responsible are facultative pathogens that cause an ascending infection (de Winter et al. 1995). When the animal's immunity is poor and the infection pressure high, these bacteria can cause disease. Both environmental and farrowing hygiene are critical in preventing VDS (Dee 1992).

VDS is commonly thought to occur more often in stalls than in loose housing. Discharge is more visible in stalls (Meredith 1991), and sows with a soiled perineal area are more vulnerable to post-mating vulvar discharge (Bara and Cameron 1994). Some loose-housing systems do, however, have extensive VDS problems; certain structural features inside a sow house, such as poorly functioning slatted floors, and inadequate hygiene in general probably predispose to VDS (Dee 1992).

Predisposing factors of VDS are many. The phase of the reproductive cycle has been found to be important in contracting endometritis and metritis (de Winter et al. 1992; 1996). Inactive ovaries are also considered a risk factor for developing endometritis (Dalin et al. 1997). Infection in the urinary tract predisposes a sow to endometritis and metritis (Bernier 1984; Bilkei et al. 1995). Other predisposing factors include restricted drinking water, lack of exercise, overcrowding and other stressors. VDS animals are a constant source of infection for other animals (Dee 1992).

Venereal transmission from the boar is considered a possible cause of spreading vaginal discharge from sow to sow (Muirhead 1986). Animals serviced with contaminated artificial insemination equipment are at higher risk of infection (Dee 1992). When bacteria were introduced into the uterus at metoestrus, gilts developed endometritis and showed vaginal discharge (de Winter et al. 1992; 1996). Post-ovulatory insemination also resulted in vaginal discharge (Kaeoket et al. 2005).

In addition to early culling of sows, VDS can cause considerable economic losses due to small litter size and impaired fertility (Dee 1992). Numerous treatment protocols have been attempted to resolve discharging-sow problems, but their success is dubious. Because the precise pathogens and the cost-effectiveness of antibiotic treatments are rarely known, it is difficult to justify these treatments (Britt et al. 1999).

A large variety of commensal micro-organisms in the genital tract makes culturing of a single microbial agent or interpretation of a mixed culture challenging (Dee 1992). A representative sample can be taken from the anterior vagina or from the uterus using a speculum with a long guarded swab, without the interference of the rich bacterial flora of the posterior vagina, when the swab is inserted through and beyond the speculum before the swab tip is exposed (Meredith 1981).

## **4.4 LAMENESS**

The main causes (72%) of killing of Danish sows were related to the locomotive system (Kirk et al. 2005). Thus, lameness affects animal welfare. Lameness has been claimed to be associated with reduced fertility (Penny 1980), but the association has not been established scientifically in pigs. However, it is well known among practising veterinarians that lameness is a common problem in pig herds, causing considerable economic losses.

Loose housing of sows in groups creates high demands on the locomotory system of the animals. The risk of lameness may be increased in group housing because the animals move about on slatted floors and aggressive interactions occur between animals. These fights may lead to distortion of joints, especially to damaged claws (Kroneman et al. 1993a). Moreover, high-ranked sows, which are typically multiparous and thus the largest, may mount primiparous and smaller subordinate sows (Pedersen 2007). This can lead to leg problems for the mounted animals.

Claw lesion incidence was higher in group housing than in crates, but no relation between claw lesions and lameness was observed (Kroneman et al. 1993a). The mean herd prevalence for claw lesions in loose-housed herds with partially slatted floors was about twice as high as in herds with individual housing (Gjein and Larssen 1995a). However, lack of exercise has well-documented adverse effects on confined sows (Vestergaard 1984).

Transferring to group housing has changed the nature of locomotory problems. Sows in cages become lame at the final stage of pregnancy, while sows kept in pens with an electronic sow feeder show symptoms during initial introduction and when animals are mixed (Anil et al. 2005).

## **4.5 ACUTE-PHASE PROTEINS (APPs)**

The acute-phase response is a non-specific early response to infection and inflammation. During this response, hepatic production of acute-phase proteins (APPs), e.g. haptoglobin (Hp) and C-reactive protein (CRP), is elevated (Baumann and Gauldie 1994). APP measurements have been suggested to be useful as diagnostic and prognostic aids and also as a means of monitoring the health status of pigs (Eckersall et al. 1996; Heegaard et al. 1998; Petersen et al. 2004). Research has so far focused mainly on young pigs. Lamé finishing pigs showed elevated Hp concentrations (Petersen et al. 2002a; 2002b). Mastitis metritis agalactia (MMA) syndrome has been shown to result in acute-phase response (Bilkei and Bolcskei 1993; Kostro et al. 2003; van Gelder and Bilkei 2006). However, very little information is available on the use of APPs in sows.

## **5. AIMS OF THE STUDY**

The primary aims of the study were as follows:

- 1) To determine the prevalence of herds having at least one high porcine parvovirus (PPV) titre or vulvar discharge syndrome (VDS) or lameness by evaluating randomly chosen loosely housed sows in Finland (I, IV, V).
- 2) To explore the potential association of the PPV antibody titre status of the herd, VDS or lameness with some fertility parameters (I, III, V).
- 3) To explore the association of environmental and management factors with PPV antibody titres, VDS and lameness (I, III, V).
- 4) To investigate methods for diagnosing PPV, VDS and lameness (II-V).

## 6. MATERIALS AND METHODS

The experiments are divided into three main categories: PPV (I, II), VDS (III, IV) and lameness (V).

The materials and methods for each study are described in detail in the original papers; only a brief overview is provided here.

### 6.1. SELECTION OF HERDS AND ANIMALS

Four studies (I, III-V) were carried out from December 2001 to May 2002 in 21 randomly chosen sow herds in Southern Finland. The original study population included 151 sow herds with loose-housed dry sows and gilts that participated in the Finnish National Litter Recording Scheme conducted by the Finnish Animal Breeding Association. These animals were situated within 150 km of the university clinic in southern Finland. The animals were mostly crossbred (Yorkshire x Landrace), with some pure Landrace and pure Yorkshire sows also included

We conducted a pilot study with seven farms and found that four of the farms (57%) had at least one animal with a high PPV titre, and on these farms 20-45% of the animals sampled had a high titre. Based on the 57% prevalence of PPV infection in herds, a sample of 21 herds was required for the estimated prevalence to be within 20% at the 95% confidence level (DiGiacomo and Koepsell 1986) (I). We calculated that 11 blood samples needed to be taken on each farm to find at least one high PPV titre with an estimated prevalence of 25% and a confidence level of 95% (DiGiacomo and Koepsell 1986) (I, II).

The prevalence of VDS in the pilot study was 1.1% (Oravainen et al. 2002) (III, IV). To study VDS (III, IV), we concluded that at least 15 farms were required for accurate evaluation, with an estimated prevalence of 1% and a maximum tolerable error of 0.05 (DiGiacomo and Koepsell 1986). In addition, in studies III and IV, we looked for case animals in so-called VDS problem herds because the prevalence of VDS was low. Five farms with vulvar discharge problems were subsequently identified with the help of health care veterinarians of the slaughterhouses and visited to obtain additional discharging animals. These five farms yielded ten VDS animals.

Of the animals in the pilot study, 5.7% were lame (Heinonen et al. 2002). Based on the pilot study and a maximum tolerable error of 0.1, an estimated 21 herds were needed to calculate the prevalence of lameness (DiGiacomo and Koepsell 1986) (V).

All animals meeting the following criteria were included in the study: gilts selected for breeding (~ 6 months old), sows and gilts 0-30 days after mating, lactating sows beyond 5 days post-partum and sows between weaning and mating (III-V). In Study I, all sows and gilts old enough to be mated were included on the same farms. One control animal displaying no clinical signs of illness was selected for each case animal and matched for farm, cycle phase and parity (III, IV) and breed (V).

Study II took place on four farms. On three farms, a group of 10 gilts was selected, and on one farm 9 gilts were available from three groups. Ten sows were sampled on each farm at the beginning and the end of the study. All of the herds were classified as specific pathogen-free (SPF) herds.

Table 1. Summary of animals and herds in Studies I-V. Twenty-one herds in Studies I and III-V were exactly the same herds. In Studies III and IV, five VDS problem farms were also included.

Study	Median of animals/herd	N of herds	Total N of study animals	Median of study animals/herd
I	70 (27-130)	21	247	12 (10-16)
II	115 (60-180)	4	39	10 (9-10)
III	85 (27-850)	26	799	27.5 (7-75)
IV	85 (27-850)	26	799	27.5 (7-75)
V	70 (27-130)	21	646	29 (12-76)

## 6.2 MANAGEMENT AND INSPECTION OF ANIMALS

The housing systems of dry sows on these 21 farms, assessed by type of flooring and use of bedding, were divided into two categories: 1) straw (N=4) and sawdust (N=5) composting systems (deep litter) and 2) concrete floor systems with little (N=1) or no (N=0) bedding and partially (N=8) or totally (N=3) slatted floors. The total area per animal was measured: mean 3.05 m<sup>2</sup>/sow (range 2-3.85). Feeding type was recorded: 19 herds had individual feeding cages, one had a feeding station and one had troughs with dividers. The group size varied from 6 animals in a concrete floor system to 20-30 sows in deep bedding. The breeding was done with both AI (median 53%, range 26-100%) and natural mating. Many farmers used both methods with the same animal and during the same heat.

One year before the commencement of the study, the randomly chosen herds (N=21) had the following history of reproductive performance (mean  $\pm$  SD): farrowing rate of 71.9  $\pm$  13.4%, repeat breeding rate of 20.9  $\pm$  8.4%, gestation length of 116.4  $\pm$  0.7 days and farrowing interval of 169.8  $\pm$  17.3 days. These reproductive performance figures are slightly lower than the mean values in the National Litter Recording Scheme of the Finnish Animal Breeding Association (later Scheme) (non-returning rate about 5% lower) (Anna Häkkinen, personal communication; Peltoniemi et al. 1999a).

Of VDS problem farms, one had a concrete floor system with little bedding, two had partially slatted floors and one had totally slatted floors. On the largest problem herd, all samples were taken from a breeding department with animals in breeding cages. Information about the total area per animal was not available for two farms, but on three farms it was 2.04-3.4 m<sup>2</sup>/sow. All problem farms had feeding cages.

Owners provided us with information on use of roughage for sows. On three out of 21 randomly selected farms, sows received no roughage, likewise on one of the VDS problem farms. Owners also informed us whether dry sows were confined to a cage at



any time (days) after breeding. Seven out of 21 randomly selected farms and 4 out of 5 VDS problem farms confined sows after breeding.

Information on the parity and breed of the animal as well as on the reproductive status was obtained from the farmer. The Scheme provided us with the summary of the herd's fertility data for each herd (non-return rate, re-breeding interval, litter size) for the period of one year prior to the farm visit (I). Information about consequent breeding, farrowing and culling of animals was gathered from the central databank throughout the subsequent production cycle (III, V). The body condition score of the animal was estimated on a scale of 1-5 (1=very thin, 5=very fat) (Ritter et al. 1999) (III-V). Cleanliness of the animal's perineal area was evaluated with three categories: clean, slightly dirty and very dirty (III).

Two veterinarians clinically inspected the animals for traces of discharge by opening the animals' vulvar lips while the animals stood or walked. If discharge was seen in the vulvar or perineal area, the sow or gilt was classified as a case (III, IV). The lameness (V) of an animal was assessed (sound=no limping, slightly lame=slight limping or stiffness, severely lame=puts limited weight on the foot or does not put foot on the ground) while she walked on a hard surface at least 10 m. Lameness included all those with at least one slightly or severely lame leg. Diagnosis for the lame limb was done clinically by thorough inspection and palpation of the entire limb, including the claws.

In VDS studies (III, IV), a disinfected vaginoscope (diameter 35 mm) was inserted into the vagina by parting the vulvar lips. Vaginoscopic examination, performed by the same veterinarian for all animals, was scored as positive if any of the following signs were observed: reddening of the cervical area or vaginal mucosa or excessive vaginal contents.

## 6.3 PPV VACCINATIONS

### 6.3.1 Basic data about vaccinations

Each animal's parity was recorded. The owner provided us with the following data: parvovirus vaccine used, storage of vaccine, vaccinator, usage of previously opened vaccine vials and vaccination programme used. All farms used an inactivated PPV vaccine (I).

### 6.3.2 Experimental design

A combination vaccine of inactivated PPV and inactivated *Erysipelothrix rhusiopathiae* with aluminium hydroxide as an adjuvant was used (Nordpremum plus<sup>tm</sup>, Pharmacia Animal Health AB, Sweden). All of the farmers routinely vaccinated their herds. They injected 2 ml of the vaccine intramuscularly 5-10 cm behind the ear of the gilt using an 18G needle (40 mm length). The recommended official vaccination programme against PPV was followed for the gilts: the first injection at the age of 6 months and a booster 3-4 weeks later. After this, sows should be vaccinated about 2 weeks before mating

(Anonymous 2002). Vaccine vials were stored in the refrigerator until use, and vials were used only immediately after opening, as recommended by the manufacturer (II).

## 6.4 SAMPLING

### 6.4.1 Blood samples

Blood samples were drawn from the *vena coccygea* or *vena saphena*. Samples were transported in a cool box within 4 h to the laboratory for storage in the refrigerator until centrifuged the next day. The serum was stored at -18°C until analysed in the virus diagnostic laboratory of the Evira (I, II) or in the laboratory of the Helsinki University Saari Clinic (III, V).

In the study evaluating the prevalence of high PPV titres (I), blood samples were drawn from 11-16 sows or gilts old enough to be mated per herd. Animals sampled were the ones that were sampled also for APP diagnostics in Studies III and V. If additional animals were needed, convenience random sampling was used. The median parity of the sampled sows per herd varied from 0 to 6. In the study on parvovirus antibodies in vaccinated gilts (II), blood samples were obtained from nursing sows just before the booster on each farm at the beginning of the study and simultaneously with the last samples collected from the gilts at the end of the study to determine whether PPV was endemic in the herds. Herds were classified as endemically infected with PPV since those samples revealed several high HI titres above vaccination level (1:1024-1:8192). Blood samples from gilts were taken four times: sampling #1 occurred 0-8 days before the first vaccination, sampling #2 at 2-3 weeks after the second vaccination, sampling #3 at mid-pregnancy and sampling #4 after farrowing and just before weaning.

### 6.4.2 Bacteriological and cytological samples

To prepare the VDS case and control animals for sampling and vaginoscopy (III, IV), the vulvar lips were washed three times with polyvidone-iodine solution (Betadine®, Leiras, Finland), rinsed with water and wiped five times with gauzes saturated with chlorhexidine (Klorhexol®, Leiras, Finland). A bacteriological sample was taken through the vaginoscope from the anterior vagina or the caudal part of the cervix with a guarded swab, and a cytological sample with an unguarded swab. The bacteriological sample was placed in a transport medium (Transpocult®, Orion Diagnostica, Finland), and the cytological smear was spread across the slide and air-dried immediately.

## 6.5 LABORATORY ANALYSES

### 6.5.1 Haemagglutinin inhibition (HI) test

Antibodies against parvovirus were detected with an HI test (Joo et al. 1976). Animals were considered to have low antibody levels when HI titres were  $\leq 1:512$ ; titres  $> 1:512$  were deemed high (Neuvonen et al. 1979). Evira's diagnostic guidelines for PPV viruses are as follows: antibody titres  $\leq 1:8$  represent an animal that has not seroconverted, 1:16-

1:512 represent intermediate seroconversion and titres beyond this represent a high level of antibodies (Anonymous 2007).

#### 6.5.2 Enzyme-linked immunosorbent assay (ELISA)

Competitive ELISA (PPV-Ab Svanovir®, SVANOVA, Uppsala, Sweden) (II) was used. The cut-off values of the ELISA test as given by the manufacturer and based on the degree of inhibition of the antibody-antigen complex formation are <50% negative, 50-80% positive and >80% strongly positive.

#### 6.5.3 Pathology

Reproductive and urinary tracts of slaughtered gilts as well as mummified and stillborn piglets were sent to Evira for post-mortem examination to determine the cause of reproductive failure (II).

#### 6.5.4 Bacteriology

The bacteriological samples were cultured in the laboratory within 4 h of the sampling on trypticase soy agar with 5% horse blood (TSH, Biomerieux, France) at +37°C both aerobically and anaerobically. On aerobic cultures, a susceptibility test for the antibiotics recommended in VDS cases (Peltoniemi et al. 2003) was performed using a disc diffusion test with sulpha-trimethoprim 245.2 µg and ampicillin 33 µg. The anaerobic cultures were read after 4-5 days of incubation. Evira diagnosed all significant anaerobic bacteria and tested susceptibility using the disc diffusion test with sulpha-trimethoprim 25 µg and ampicillin 10 µg (IV).

#### 6.5.5 Cytology

The cytological samples were fixed with methanol, stained with methyl violet and analysed microscopically. The slide was examined under a microscope (x100), and the number of leucocytes in 10 fields was counted. The samples were divided into four groups: no leucocytes (0), 1-10 leucocytes (+), >10 individually occurring leucocytes (++) and large clumps of leucocytes (+++). Counting was performed independently by two persons. In case of disagreement (16% of slides), the average count was taken. The leucocyte count was considered negative for results of 0 and +, and positive for results above this level (IV).

#### 6.5.6 APPs

CRP was analysed with a solid-phase sandwich immunoassay kit for porcine CRP (Tridelta Ltd., Ireland). Serum Hp was determined using the bovine haemoglobin-binding assay described by Makimura and Suzuki (1982), with the modification that tetramethylbenzidine (0.06 mg/ml) was applied as a substrate (Alsemgeest et al. 1994) adapted for microtitration plate use. The assay was calibrated using a reference porcine

acute serum sample provided by the European Commission Directorate General Research Concerted Action Group (project number QLK5-CT-1999-0153) (Skinner 2001) (III,V).

## **6.6 STATISTICAL ANALYSES**

Statistical analysis was performed using Stata software (Stata Corporation, College Station, TX, USA), version 8.0 (I, II, IV), 8.1 (V), 8.2 (III), SPSS for Windows (SPSS INC., Chicago, IL, USA), version 10 (II, III) and Statistix Netware (Analytical Software, Tallahassee, FL, USA), version 1 (III).

### **6.6.1 Unconditional analyses**

#### **6.6.1.1 Parametric tests**

Potential associations between high PPV antibody titres and some herd fertility parameters (non-return rate, rebreeding interval, litter size) were examined with Student's t-test since they were normally distributed. (I).

#### **6.6.1.2 Non-parametric tests**

Unconditional analyses of relationships between PPV titres (I), VDS (III), fertility (III, V), lameness (V) and potential risk factors were performed using chi-square test for dichotomous variables.

The reproductive results of the gilts with high HI titres by mid-pregnancy (during the first three samplings) were compared with those of animals having low titres by using Fisher's exact test (II). Bacteriological, cytological and vaginoscopic results between case and control pigs were also compared with Fisher's exact tests (IV).

The potential association between ELISA and HI tests was investigated with Spearman's correlation and the kappa quotient. The latter was calculated in categories (Martin et al. 1987). (II).

The association of APPs with VDS, bacteriological growth and lameness was evaluated using a median test (III).

The association between parity and VDS was evaluated with a Mann-Whitney test (IV).

McNemar's chi-square test was used to determine any associations between bacteriological, cytological and vaginoscopic examination results and vulvar discharge (IV).

Statistical analysis of the bacteriological cultures of VDS animals and their controls was carried out by using a matched case-control test (IV).

APP comparisons between VDS animals (III) or lame animals (V) and their controls were performed using Wilcoxon signed-rank test.

#### 6.6.2 Model building strategy

Unconditional analyses of relationships between PPV titres (I), VDS (III), fertility (III, V), lameness (V) and possible risk factors were performed with a chi-square test for dichotomous variables and a logistic regression for categorical variables. Variables with a p-value  $\leq 0.15$  were included in the multivariable model.

The multivariable analysis in Study V was carried out using a generalized latent and linear mixed model, with herd set as the random effect. A manual backward stepwise elimination procedure with a cut-off level of 0.05 was used.

The final multivariable analysis in Studies I and III was performed using a generalized estimating equation (GEE) population-averaged model (Dohoo et al. 2003) with a manual backward stepwise elimination and a cut-off level of 0.05. This model takes into account the correlation between pigs on a single farm. Farm was set as a group variable because the data were strongly clustered.

The effect of VDS on farrowing in Study III was subsequently observed with deletion diagnostics (Preisser and Qaqish, 1996). Each farm in turn was dropped from the analysis and then each VDS animal.

## 7. RESULTS

This section provides an overview of the main results. All results are described in detail in the original publications.

### 7.1 STUDIES ON PPV

#### 7.1.1 PPV prevalence study (I)

Altogether 247 animals were sampled. Seventeen farms (81%) had at least one animal with a high titre, with 44% of all animals having high titres (Table 2).

Table 2. Numbers of animals according to three levels of HI titres against PPV in 21 loosely housed herds and three fertility figures of each herd for the year prior to the visit.

<i>Farm</i>	<i>Titres ≤1:8 N</i>	<i>Titres 1:16- 1:512 N</i>	<i>Titres &gt;1:512 N</i>	<i>Non-return rate %</i>	<i>Rebreeding interval</i>	<i>Litter size (piglets born alive)</i>	<i>Vaccination programme</i>
1, N=13	0	9	4	87.9	28	10.9	shortcomings
2, N=11	2	9	0	70.8	29	11.0	shortcomings
3, N=11	0	7	4	62.8	26	12.5	shortcomings
4, N=11	1	7	3	87.0	32	11.0	shortcomings
5, N=12	0	1	11	72.0	30	7.3	shortcomings
6, N=13	0	3	10	83.8	28	10.0	recommended
7, N=12	2	7	3	76.8	25	10.2	shortcomings
8, N=12	1	6	5	71.2	29	11.7	shortcomings
9, N=11	1	5	5	90.6	26	11.9	recommended
10, N=12	0	1	11	74.6	30	11.0	shortcomings
11, N=11	0	4	7	67.5	32	10.7	recommended
12, N=12	0	5	7	84.7	27	10.9	shortcomings
13, N=12	0	4	8	77.4	25	11.1	recommended
14, N=11	0	9	2	82.0	23	10.7	shortcomings
15, N=11	1	10	0	86.6	27	10.7	recruitment animals only
16, N=11	0	11	0	81.6	27	11.8	shortcomings
17, N=16	1	9	6	71.4	24	11.1	recommended
18, N=12	0	2	10	77.7	24	11.0	shortcomings
19, N=11	2	8	1	85.2	29	11.5	shortcomings
20, N=10	2	8	0	96.4	44	10.0	recruitment animals only
21, N=12	0	0	12	81.5	30	10.2	not vaccinated
(Sum)	13	125	109	80.3*	28.3*	10.8*	
(mean*)							
sd				7.6	4.4	1.0	

The vaccination programme was carried out according to official recommendations at 5 farms, but 16 farms had shortcomings in their programme (including 3 farms that did not vaccinate at all). The most common shortcoming was that the gilts were vaccinated only once before mating (7 herds). Interaction between parity and vaccination programme shows that the effect of programme depends on parity, i.e. the effect of programme on high HI titres is different in gilts than in sows with a parity of two or higher (Table 3). Of the 3 farms where no vaccines were used, 2 had only low antibody titre levels. These two farms bought recruitment gilts that were already vaccinated. All animals sampled on the third farm had high titres.

Fourteen farmers stored vaccines after use and used previously opened vaccine vials, 2 farmers did not store the vaccines since the animals were vaccinated by a veterinarian and 2 farmers used the vials only immediately after opening.

Table 3. The final GEE population-averaged model for variables associated with high (>1:512) PPV haemagglutinin inhibition titres with farm as a group variable.

Titres	OR	SE	P> z	95% CI
Herd size <70 sows				
Herd size $\geq$ 70 sows	0.2	0.1	0.009	0.04-0.6
Parity 0				
Parity 1	0.6	0.6	0.6	0.08-4.6
Parity $\geq$ 2	13.9	10.9	0.001	3.0-64.6
No storage				
Storage	3.5	2.4	0.07	0.9-13.3
No vaccination	0.8	0.8	0.9	0.1-5.4
Programme (recommended)				
Programme (shortcomings)	1.0	1.1	1.0	0.1-8.7
Programme (shortcomings)*Parity 0				
Programme (shortcomings)*Parity 1	1.1	1.3	0.9	0.1-10.8
Programme (shortcomings)*Parity $\geq$ 2	2.5	1.1	0.03	1.1-5.7

#### 7.1.2 PPV antibodies in vaccinated gilts (II)

Herds had a median of 59.0% (range 36.4-81.8%) and 36.4% (9.1-81.8%) sows with HI titres above vaccination level at the beginning and the end of the study, respectively. Thus, all herds were considered to be endemically infected with PPV.

At the beginning of the experiment, 33 of the gilts (84.6%) had negative HI antibody titres. Figure 1 shows the median values and ranges of HI and ELISA results during the samplings. The number of the gilts decreased because nine animals were culled, three because of infertility, two because of anoestrus, two because of lameness and two

because of an accident. In addition, sampling #4 was not possible for the 17 animals that had conceived early and had had sampling #3 just before weaning. Altogether 27 seronegative gilts (81.8%) in the first sampling had increased their titre level to 1:16-1:512 during the second or third samplings. Two gilts did not seroconvert and four had HI titres above 1:512. A fair agreement existed between HI and ELISA; Spearman's rho for the tests was 0.87 and kappa 0.63.

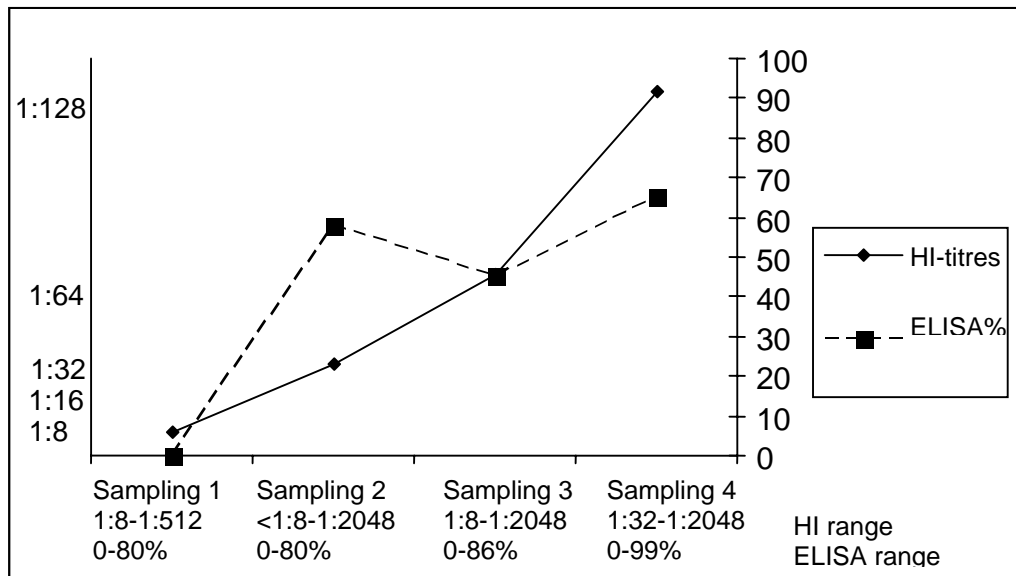


Figure 1: HI titres and ELISA percentages of 39 gilts before and after vaccinations against porcine parvovirus. Sampling 1: before the first vaccination at the age of 6 months with the booster 3 weeks later (N=39), sampling 2: 2-3 weeks after the booster (N=39), sampling 3: at mid-pregnancy (N=35), sampling 4: after farrowing but before weaning (N=13).

One stillborn piglet was found with HI titres of 1:32 in a litter with seven live piglets. Their dam had seroconverted after the first vaccination, and she had titres of 1:32 in all three samplings (#2-#4). Intake of colostrum could not be totally ruled out as a contributing factor.

## 7.2 STUDIES ON VDS

### 7.2.1. Factors associated with fertility and VDS (III)

Nine (47.4%) of the 19 VDS animals and 533 (68.3%) of the 780 non-VDS animals farrowed at the earliest opportunity after our farm visit ( $P=0.05$ ). Altogether, 542 animals (67.8%) farrowed at first chance. The culling rate showed that 19.9% of the non-VDS animals and 42% (8/19) of VDS sows were culled. Animals were observed until the next possible farrowing or selling to another herd.

The final multivariable analysis model (Table 4) for farrowing included reproductive status, vulvar discharge syndrome, use of roughage, keeping sows in cages after



breeding and the interaction term of housing system\*cage. Interaction between housing system and keeping sows in cages after breeding showed that the effect of confinement to cages is different in different housing systems, i.e. sows in concrete floor systems do not benefit from confinement as much as the sows in deep litter systems. The effect of VDS on farrowing was subsequently observed with deletion diagnostics. Farms were dropped from the analysis one at a time, followed by VDS animals. With one farm excluded at a time, the odds ratio ranged from 0.2 (CI 95% 0.08-0.7) to 0.5 (CI 95% 0.2-1.4). With one VDS animal excluded, the odds ratio ranged from 0.3 (CI 95% 0.1-0.8) to 0.4 (CI 95% 0.2-1.1).

Table 4. Final multivariable model of factors associated with farrowing after the farm visit. Analysed using a GEE population-averaged model with farm as a group effect.

	Risk factor	OR	SE	P> z	95% CI
Reproductive status	unmated gilts#				
	early pregnancy	2.9	0.6	0.000	1.9-4.4
	nursing and weaned	2.3	0.5	0.000	1.5-3.6
	or aborted sows				
Vulvar discharge	no#				
	yes	0.4	0.2	0.04	0.1-1.0
Use of roughage	no#				
	yes	2.3	0.4	0.000	1.7-3.2
Confinement to cage	no#				
	yes	3.0	1.2	0.007	1.4-6.8
Housing system	deep litter#				
	concrete/slats	1.1	0.2	0.6	0.7-1.6
Interaction term	deep litter*cage#				
	concrete/slats*cage	0.3	0.2	0.02	0.1-0.8

#=reference parameter

Variables tested with univariate analysis for an association with vulvar discharge are presented in Table 5. The initial multivariable analysis model for VDS included housing system and keeping sows in cages after breeding with farm as a group effect. None of the variables tested in the multivariable analysis remained significant.

Table 5. Univariate analysis of variables associated with VDS. %=animals with VDS.

Variable		N	%	P
Parity	0	78	2.6	
	1	215	2.8	0.9
	≥2	368	3.0	0.8
Housing system <sup>a</sup>	deep litter	173	1.2	
	concrete with little or no bedding and slatted floors	490	3.5	0.1
Reproductive status <sup>*</sup>	early pregnancy	371	2.2	
	nursing, weaned or aborted	292	3.8	0.2
BCS	1 and 2 (very thin and thin)	68	2.9	
	3, 4 and 5 (moderate, fat and very fat)	595	2.6	1.0
Cleanliness	clean	62	1.6	
	moderately clean	337	2.1	0.8
	dirty	264	4.2	0.4
Use of roughage	no	179	3.9	
	yes	484	2.5	0.3
Herd size	≤85 sows	334	2.7	
	>85 sows	329	3.0	0.8
Confinement to cage <sup>a</sup>	no	322	1.9	
	yes	341	3.8	0.1

<sup>a</sup>Variables included in the multivariable model.

\* Unmated gilts were excluded because none had VDS.

#### 7.2.2 VDS prevalence and vaginoscopic, bacteriological and cytological results (IV)

The median VDS prevalence on farms in Study IV was 0% (range 0-4.5%). In these 21 herds, nine of the 655 animals had VDS (1.4%, 95% CI 0.5-2.3%). Fourteen of the 21 farms had no VDS cases. The median prevalence on the five VDS problem farms was 14.3% (range 3.0-18.2%). As expected, these five farms had more VDS cases; 7.4% of the animals studied.

In total, 19 animals were identified with VDS. Their median parity was 2 (0-9, minimum-maximum), which was not statistically different from that of controls (1, 0-13,  $P=0.3$ ). Ten case animals (53%) showed positive results in bacterial culture, either aerobic or anaerobic, or both, while three of the control animals showed positive results (16%;  $P=0.04$ ) (Table 6).

Seventeen of the 18 cases (94%) were positive in the vaginoscopic examination (two examinations were missing, one case and its control), whereas five of the 18 controls were positive (28%;  $P<0.0002$ ). Two of the three control animals yielding a positive bacteriological sample also had a positive finding in the vaginoscopic examination.

In cytological examination, seven case animals showed positive results (37%), while control sows yielded only one positive result (5%;  $P=0.04$ ) (Table 6).

Vulvar discharge findings were compared with bacteriological, cytological and vaginoscopic examination results. VDS findings were associated with vaginoscopic examination ( $P=0.0005$ ) and with bacteriological findings ( $P=0.04$ ), but the association with cytology was weak ( $P=0.07$ ).

Table 6. Results from 19 case animals with vulvar discharge and their controls in problem (P) and non-problem herds (N). Samples for bacteriology and cytology (CYT) were taken from the anterior vagina. Vaginoscopy (VAG), + = positive, - = negative, M = missing, Non-sp. = Non-specific, Stage (days after Farrowing F, Weaning W or Heat H).

Herd/pair	Case animals				Control animals			
	BACTERIOLOGY		CYT/VAG		BACTERIOLOGY		CYT/VAG	
	Aerobic	Anaerobic		Stage	Aerobic	Anaerobic		Stage
P2/1	-	<sup>k</sup> <i>Lactobac.sp.</i> <sup>l</sup> <i>Peptostr.sp.</i>	-/+	F5	-	-	+/-	F4
N4/1	-	-	-/+	F5	-	-	-/-	F5
P5/2	-	-	-/+	F9	-	-	-/+	F8
N1/2	<sup>b</sup> <i>A. pyog.</i>	<sup>b</sup> <i>A. pyog.</i> , <sup>d</sup> <i>P. asach.</i>	+/+	F11	<sup>c</sup> <i>S. aur.</i>	Non-sp.	-/+	F11
P1/1	<sup>a</sup> <i>E. coli</i>	<sup>j</sup> <i>F.necr.</i>	+/+	F13	-	-	-/-	F12
N7/1	-	-	-/-	F22	-	-	-/-	F19
N1/1	<sup>n</sup> <i>Past.sp.</i> , <sup>a</sup> <i>E. coli</i>	Non-sp.	+/+	F31	-	-	-/-	F32
N5/2	-	-	-/+	F36	-	-	-/-	F31
P3/3	-	-	-/+	W6	Mixed	<i>Cl. perfr.</i> <sup>e</sup>	-/+	W6
P3/2	-	<sup>m</sup> <i>Prev. sp.</i>	+/+	W6	-	Non-sp.	-/-	W6
P3/4	-	-	+/+	W6	-	-	-/-	W6
N3/1	<sup>a</sup> <i>E. coli</i>	Mixed	M/M	H2	-	-	-/M	H2
N5/1	-	-	-/+	H4	-	-	-/-	H6
P3/1	-	<sup>h</sup> <i>Porph. sp.</i>	-/+	H9	-	-	-/-	H9
P2/2	-	<sup>g</sup> <i>A.suis</i>	+/+	H11	-	-	-/-	H8
N6/1	-	-	-/+	H13	-	-	-/-	H13
N2/1	<sup>i</sup> CNS	<sup>i</sup> CNS	+/+	H16	-	-	-/-	H24
P5/1	-	-	-/+	H17	-	-	-/+	H12
P4/1	<sup>f</sup> Colif. sp.	Non-sp.	-/+	H22	-	-	-/+	H22

<sup>a</sup>*Escherichia coli*, <sup>b</sup>*Arcanobacterium pyogenes*, <sup>c</sup>*Staphylococcus aureus*, <sup>d</sup>*Peptostreptococcus asacharolyticus*, <sup>e</sup>*Clostridium perfringens*, <sup>f</sup>Coliform sp., <sup>g</sup>*Actinobaculum suis*, <sup>h</sup>*Porphyromonas sp.*, <sup>i</sup>Coagulase-negative staphylococci, <sup>j</sup>*Fusobacterium necrophorum*, <sup>k</sup>*Lactobacillus sp.*, <sup>l</sup>*Peptostreptococcus sp.*, <sup>m</sup>*Prevotella sp.*, <sup>n</sup>*Pasteurella sp.*

## 7.3 STUDY ON LAMENESS

In all, 57 animals (8.8%) were lame, and of these 22 (3.4%) were severely lame. A median of 7.0% (0-27.3%) of the animals in a herd were lame.

Of the 646 animals, 68.3% became pregnant about the time of the herd visit. Lameness was not associated with fertility in univariate analysis ( $P=0.9$ ). In the multivariable analysis, only parity and use of roughage remained significant factors. Of the total number of animals, 18.4% (119) were culled before next farrowing. Of the 57 lame animals, 22.8% were culled, 7% because of lameness and 10.5% because of fertility problems. Among healthy animals ( $N=589$ ), the respective figures were 18%, 2.9% and 8.5%.

The following factors were included in the multivariable logistic regression model for lameness with herd as a random effect: breed, type of loose housing in the herd (with or without slatted floors) and the interaction term area\*cage. The final multivariable model is shown in Table 7. Similarly, the type of loose housing, use of roughage and area\*cage were included in the model for severe lameness, but only the type of housing remained a significant factor in the final model (Table 7).

Table 7. Final multivariable logistic regression models with farm as a random effect for the animal being A) lame and B) severely lame in 21 randomly selected loose-housed herds.

	Variable	Parameter	b	SE (b)	P	OR (95%CI)
A. Model for being lame	Breed	Constant	-3.03	0.31	>0.001	
		Landrace				
		Yorkshire	0.99	0.38	0.01	2.7 (1.3, 5.8)
		Crossbred	0.16	0.32	0.6	1.2 (0.6, 2.2)
	Housing	No slatted floors				
		Slatted floors	0.70	0.31	0.02	2.0 (1.1, 3.7)
B. Model for being severely lame	Housing	Constant	-4.23	0.50	<0.001	
		No slatted floors				
		Slatted floors	1.32	0.55	0.02	3.7 (1.3, 11.2)

## 7.4 APP RESULTS

APP concentrations were not significantly higher in VDS animals than in controls (Table 8). Hp and CRP were not associated with bacteriological growth ( $P=0.7$  and  $P=0.06$ , respectively).

APP concentrations were higher in lame animals, especially in severely lame animals, than in controls (Table 8).

Table 8. Hp (g/l) and CRP (mg/l) of A) lame, B) severely lame and C) VDS and control sows, expressed as medians (range). One clinically healthy control animal matched by farm, parity, reproductive status (A,B,C) and breed (A,B) was selected for each case animal.

	Case animals	Control animals	P
A. Lame, 57 animals			
Hp (N=45)	2.2 (0.3-4.3)	1.8 (0.9-3.9)	0.09
CRP (N=51)	60.8 (0.5-341.1)	26.3 (2.5-201.5)	0.006
B. Severely lame, 22 animals			
Hp (N=18)	2.5 (0.5-4.0)	1.8 (1.2-2.8)	0.02
CRP (N=20)	116.2 (13.5-250.0)	33.6 (3.7-162.8)	0.0004
C. VDS, 19 animals			
Hp (N=14)	2.5 (1.3-3.1)	2.3 (0.5-4.3)	0.6
CRP (N=15)	30.3 (3.3-171.3)	25.9 (3.3-361.1)	0.7

## 8. DISCUSSION

### 8.1 GENERAL DISCUSSION

Animal welfare has been a significant factor in the decision-making for moving gestating sows to loose-housed systems. Our study gives a glimpse of the problems that can occur with poor loose-housed systems. Dirty floors without bedding are slippery and predispose to ascending infections of the reproductive tract. Not providing sows with roughage impairs their reproductive performance. Slatted floors are strongly associated with lameness. Well-designed and -maintained deep litter systems appear to be the best option for preventing lameness and VDS, whilst providing sows with high-welfare conditions.

When new housing systems are designed, several criteria must be taken into account, e.g. productivity, labour requirements and management, welfare and health, and investment costs (den Hartog et al. 1993). However, modern group housing systems are relatively new developments and are likely to improve rapidly as efforts are directed toward controlling such problems such as aggression, claw disorders, and manageability of sows. In Finland, these improvements are now underway.

Mortensen (1990) reported that labour input for groups was high at the beginning of the switch to a new system, but decreased later; no real differences between the individual and group housed sows were noted. He reported that 5% of the sows were unable to adapt to the group housing system or had to be removed because of fighting.

Hierarchy positions in groups of sows are a factor to be considered in management, including the method of chosen for feeding (Arey and Edwards 1998). Brouns and Edwards (1994) found that low-ranking sows gained less weight than high-ranking sows in the floor-fed pens, but not in the ad libitum pens. By changing their feeding strategy, low-ranking sows in an ad libitum feeding system could achieve comparable intake with higher-ranking animals.

When sows are kept under conditions where the social pressure is high, e.g. due to limited space and/or resources, the social stress experienced particularly by subordinate individuals may inhibit sexual motivation during oestrus. For example, fear reduces the sexual motivation during mating and during sexual interactions amongst sows within a group, and fear may thus inhibit their chances of reproductive success (Pedersen 2007). However, achieving equally good reproductive performance in group housing systems as in systems with individual housing is possible, provided that good management is practised (Arey and Edwards 1998).

Housing and management can influence the incidence of lameness and foot lesions by limiting the number and type of movements that the pigs make (Kroneman et al. 1993b). Mortensen (1990) reported considerable problems with leg injuries and vulva biting. Under natural conditions, the ground is usually soft and damp. When pigs are kept indoors, the flooring should be non-slippery, non-abrasive and provide stability for the moving animal.

In a study on validation of gestating sow housing, the experts identified tethering and individual housing in stalls as low-welfare systems. The group of mid-welfare systems contained indoor group housing systems and an individual housing system with additional space and substrate. The five best systems were all systems with outdoor access and the provision of some kind of substrate such as straw (Bracke et al. 2002).

## 8.2 PPV

The PPV prevalence found in Finland (81% of farms and 44% of animals sampled) is comparable with that of many other countries, including Canada, Hungary, the UK and Italy (Gagnon and Dulac 1979; Moscari et al. 1983; Robinson et al. 1985; Foni and Gualandi 1989; Nash 1990). However, only in the survey conducted by Nash in the UK in 1990, were vaccinations widely administered. In that study, 96% of farms and 77% of animals were positive. PPV-linked problems have diminished markedly since vaccination has become more common. However, PPV is still the most important infectious cause of herd-level infertility problems in Finland. Very high (>1:2048) PPV titres are commonly found in vaccinated herds with fertility problems without any signs of other reproductive diseases.

Zeeuw et al. (2007) suggested that a field isolate of PPV exists in Germany (PPV-27a) that represents a new antigenic variant or type of PPV, and vaccines based on the established vaccine viruses may not be fully protective against this field isolate. Moreover, the ubiquity of PPV may indicate that some pigs remain infected and shed the virus periodically. However, shedding after the acute phase of infection has not been demonstrated (Johnson et al. 1976). Gradil et al. (1990) suggested that pigs infected with PPV *in utero* may be persistently infected, but the likelihood of shedding to contact animals is minimal.

The present results show that herd size and parity are by far the most significant determinants of PPV titre status of the herd. The older sows had higher titres regardless of the vaccination programme (recommended/shortcomings). This might be due to their having lived longer, thus increasing the chance of contracting a natural infection. Larger farms more often have compartments, and therefore, the infection does not spread as easily. However, Cutler et al. (1983) did not find any relation between PPV infection and herd size. Storage of vaccine vials after use seemed also to be a risk factor for high titres. Storage of vials against manufacturers' recommendations is most probably caused by farmers' stinginess, which may lead to marked economic losses if a PPV outbreak arises.

Our study shows that in endemically infected herds most gilts had not seroconverted by the age of 6 months, i.e. at the time of breeding. None of the gilts had the high HI titres indicative of natural infection at that time. This is in agreement with the study of Sorensen et al. (1988), in which only 7% of 6- to 7-month-old gilts had seroconverted. In our study, on-field vaccination resulted in a consistent increase in humoral immunity not exceeding the antibody level of 1:512 in the majority of gilts in all herds examined. The results of HI and ELISA agreed moderately, supporting the findings of experimental studies by others (Hohdatsu et al. 1988; Westenbrink et al. 1989).

As our results show, repeat breeders, gilts culled due to infertility or gilts with small litters were no more likely to have high HI titres than their control animals. However, two gilts that did not seroconvert before farrowing and farrowed small litters most likely suffered from PPV. The small litters of these gilts and the PPV antibodies found in the other gilts piglets' serum support this observation. Castro et al. (1992) also found that 6% of gilts vaccinated twice remained seronegative until farrowing, but reproductive performance was not reported separately for these animals.

Infections that are widespread, like PPV, need to be controlled with regular procedures. The goal is to keep the majority of the population immune to the disease and to limit the prevalence of the organism so that susceptible animals can profit from the herd-level immunity. However, if only gilts are vaccinated or vaccination is irregular, gradually the proportion of older sows that become susceptible to PPV increases and the risk of a PPV epidemic rises (Huysman et al. 1992).

Interpretation of HI titres is very complicated in vaccinated herds because high HI titres, especially when interpreting an individual sample, do not necessarily indicate PPV-linked problems in an animal. A proper PPV epidemic diagnosis should also include other factors. Mummified foetuses or stillborn piglets should be sent to a diagnostic laboratory for PPV antibody or virus detection. In addition, other symptoms in the herd, e.g. conception failure, delayed return to oestrus, embryonic and foetal death with resorption, mummification and small litter sizes, stillborn piglets, neonatal death and abortion, should be consistent with PPV infection. Other reproductive diseases should also be excluded; fortunately, in Finland, none besides VDS exist. The vaccination programme should also be checked. Should there be shortcomings in the programme, measures to investigate herd-level PPV infection are warranted.

According to a recent study, PPV-NS1 ELISA (PPV non-structural protein 1 ELISA) can differentiate between PPV-infected pigs and inactivated PPV-vaccinated pigs and could be applied in disease diagnosis and surveillance. Serum conversion against NS1 was first detected 10 days after infection, and antibodies were detectable up to half a year post-infection (Qing et al. 2006). The main means of protection against PPV infection is humoral immunity, but the possibility of cellular immunity playing a role in controlling PPV reinfection remains open (Ladejkaer-Mikkelsen 2002).

### **8.3 VDS**

VDS decreased farrowing rate, which is supported by earlier experimental findings (de Winter et al. 1995). Providing animals with roughage increased odds of farrowing. A similar result was found in a large data set of 1298 herds (Peltoniemi et al. 1999b). The use of relatively young sows in the present study may have amplified the beneficial effect of roughage on reproduction since young sows may benefit more from abundant amounts of fibre-rich feed (Peltoniemi et al. 1999b). The association of age with fertility was also upheld, i.e. gilts have lower fertility (Hurtgen and Leman 1980; Peltoniemi et al. 1999a). Farrowing rates in our results were relatively small due to the way in which they were calculated; the sow was considered a success if she farrowed at the next opportunity after the farm visit. Overall, fertility parameters for the farms one year prior to



the farm visit were also lower than the mean values in the Scheme. This might be due to a relatively small number of herds and a large variation between herd performances.

Keeping sows in cages after breeding made them more likely to farrow at first opportunity. These findings are in agreement with those of Peltoniemi et al. (1999b). Moreover, Lynch et al. (1984) found that group housed sows had poorer reproductive performance than individually housed individuals because of a combination of failure to show oestrus, lower conception rate and loss through injuries due to fighting. In deep litter systems, confinement to cages was even more favourable than in concrete or slatted systems. This might be due to confinement overall being favourable, and in concrete and slatted floor systems, animals preferring to lie in crates rather than walk on slippery surfaces. In deep litter systems, sows prefer to use the loose area and do not achieve the benefit from staying in individual crates for the critical period of pregnancy.

All five problem farms had a concrete housing system with partly or totally slatted floors and little or no bedding. Due to the small number of problem farms, this descriptive finding of the impact of housing systems on VDS was not significant in the multivariable model.

Inspecting the perineal area of the sow only once is not a very dependable method to diagnose VDS. Although the most obvious clinical sign in bacterial genital infections is vulvar discharge (Meredith 1991), sows only show these signs intermittently. Thus, we may have failed to identify VDS in some animals. Post-mortem examinations of animals culled because of VDS can be valuable, but if the infection is not in an acute phase, the findings may be minimal. Furthermore, contamination during the slaughtering process might give the wrong impression about the organism behind the symptoms. As no gold standard exists for diagnosis of VDS, we chose visible discharge as the indicator and compared this with other tests available to see whether the results supported each other. We also wanted to identify acutely infected animals. In practice, animals can be selected for further examination after the herdsmen observe discharging animals over a period of time.

A vaginal speculum can be helpful in detecting infections in sows with no apparent discharge (Meredith 1991). In problem herds, vaginoscopy can be used as the primary tool for identifying animals for sampling. Using vaginoscopy, a more accurate diagnosis is possible, also indicating where in the urogenital system the discharge originates (Meredith 1991).

Only 10 out of the 19 case sows were bacteriologically positive. Different bacteria have been isolated from the anterior vagina of normal sows (Bara et al. 1993). Similar bacteria are present in clinical cases of VDS (Winter et al. 1995). A transient microbial flora exists in the anterior vagina, with microbes having potentially pathogenic properties. In our study, not all isolates were likely to cause significant bacterial infections. Some of these VDS cases could have been physiological and part of the normal genital flora.

The significance of anaerobic bacteria, except for *Actinobaculum suis* (Woldemeskel et al. 2002), is poorly understood in sows, but these bacteria may contribute to treatment failures. *Porphyromonas asacharolyticus* is prevalent in the urogenital and intestinal tracts, and it is considered to be a significant pathogen in humans (Jousimies-Somer et al. 1999). *Porphyromonas levii* causes very serious vulvovaginitis in cows (Elad et al. 2004). *Fusobacterium necrophorum* is known to occur when the epithelium is damaged

by trauma, viral or bacterial infection or maceration (Timoney et al. 1992). In addition, prevotellas (Leser et al. 2002), lactobacilli and peptostreptococci (Robinson et al. 1981) have been found in pig faeces. Thus, poor hygiene might contribute to the presence of these bacteria in the vagina.

Culling of repeat-breeding, discharging animals has been recommended (Dial and MacLachlan 1988). Sows in our study were not treated with antibiotics according to authors' recommendations. However, if treatment is used, antimicrobial sensitivity testing is advised. In this study, almost half of the isolates were resistant or intermediate to two of the recommended antimicrobial agents in Finland (Peltoniemi et al. 2003). These results are consistent with other studies. Bilkei et al. (1995), for instance, studied the urine of puerperal VDS sows and found *E. coli* and streptococci, which were resistant to neomycin, sulpha-trimethoprim, chloramphenicol and tetracycline. Treating sow herds with antimicrobials without proper testing for antimicrobial resistance can lead to poor treatment results. Testing for antimicrobial resistance appears to be more relevant at the herd level, especially when planning treatment strategies in herd health programmes.

VDS was found to be a rare disease at the population level. However, on problem farms, this disease can be disastrous to the farm's economy and very frustrating for the farmer. Thus, health care veterinarians should have a clear understanding of how to identify and treat the problem at herd level. Although treatment of VDS with antimicrobial agents appears indicated in certain cases, as a rule of thumb the emphasis should be on prophylaxis rather than treatment (Dial and MacLachlan 1988; Dee 1992). Concentrating on elimination of VDS risk factors is much more satisfactory from the herd health perspective than repeated attempts at treating VDS.

## 8.4 LAMENESS

The prevalence of lameness was quite high; almost every tenth animal was diagnosed as lame, and yet the owner had not noted it or reacted to it. Similar findings were observed in a study on sows in late pregnancy, where the mean prevalence of lame sows was 9.6% (range 0.8-20.1%) (Holmgren et al. 2000). In herds with loose housing of dry sows on partly slatted floors, the mean prevalence of sows with hind leg lameness was 13.1% (Gjein and Larssen 1995b). In an epidemiological study (Lingaas and Ronningen 1991), the incidence rate of hoof or joint diseases was only 0.37-1.71 per farrowing, implying that the disease is either uncommon or not treated.

Our finding of slatted floors being a risk factor for lameness has been documented earlier. The straw bedding system apparently results in fewer hoof lesions than a system with solid concrete floors and partly slatted floors (Ehlorsson et al. 2002). In sow pools, an increasing prevalence of claw and leg injuries was found with increasing percentages of sows kept in concrete pens compared with straw bedding (Holmgren et al. 2000). Mouttotou et al. (1999) studied the effect of floor type on foot lesions of finishing pigs. They found that pigs kept on soft floors had a lower prevalence of sole erosions, heel erosions and heel flaps and a higher prevalence of white line lesions, false sand cracks, wall separations and toe erosions than pigs kept on solid concrete floors.

Also playing a role in the development of lesions are the slipperiness, abrasiveness, surface profile and cleanliness of the floor as well as the ability of the animals to move and thus avoid fighting (Kroneman et al. 1993b).

Yorkshire animals were more likely to be lame, in contradiction to earlier results of Landrace pigs being more likely. In a study undertaken in Finland (Peltoniemi et al. 1992), the main complaint about Landrace breeding boars was leg weakness, whereas most of the complaints involving Yorkshire boars were regarding an unwillingness to mate. Landrace boars were also more severely affected than Yorkshires concerning osteochondrosis (OC) in most of the localities investigated (Jorgensen and Andersen 2000).

Our study did not confirm our hypothesis based on the pilot study (Heinonen et al. 2002) that lame animals would be unlikely to become pregnant. However, Penny (1980) claimed that an association exists between lameness and reproductive failure. In cows, lameness after calving has been associated with significantly increased intervals from calving to conception (Collick et al. 1989). In addition, English and Edwards (1999) described that lameness, associated with poor environment, is a stressful situation leading to impaired productivity. No association has, however, been found between lameness of sows in the last month of pregnancy and farrowing results (piglets born alive, stillborn piglets and birth weight) (Kroneman et al. 1993a); however, these authors did not follow the animals until the next mating.

Every tenth sow is lame, which reveals that a high percentage of animals constantly suffer from pain when moving. Environmental factors such as slatted floors cause lameness, which can lead to culling. Thus, lameness can be regarded as one indicator of unsatisfactory housing conditions, producing decreased sow well-being and impacting farm economics.

## 8.5 APPs

APPs were higher in severely lame adult animals than in controls, which is in agreement with the results obtained with lame finishing pigs (Petersen et al. 2002a; 2002b). However, in controls, the overall level of Hp was higher in our adult animals than in other adults (Richter 1974) or fattening pigs (Heegaard et al. 1998c). In a recent study, Hp levels of piglets (2 months of age) were comparable with our results (Hiss and Sauerwein 2003). Petersen et al. (2002b) found that herd influenced Hp concentration in growing pigs. CRP values in sows of earlier studies are consistent with our findings; Turk et al. (2003), using the same method as here, obtained values from 0.8 to 35.2 mg/l. Using a turbidometric method, Kostro et al. (2003) reported values from 17.6 to 158.9 mg/l. Thus, the values seem to vary between herds and laboratories. Further research is needed on the APP levels in healthy adult pigs to obtain more accurate results.

In chronic conditions, such as arthritis, consecutive series of inflammatory stimuli occur and elevated APPs are detected (Petersen et al. 2004). The lesions that have earlier been reported to raise APPs in swine have all had severe and serious infections or inflammatory responses, e.g. *Actinobacillus pleuropneumoniae* and subcutaneously administered turpentine (Eckersall et al. 1996; Heegaard et al. 1998). In our study with a

small number of animals, conditions causing severe lameness elevated the APP levels significantly. Unfortunately, we had no information on the onset of lameness.

VDS outside the post-partum period tends to mostly consist of local infections. APP findings in this study support this conclusion. APPs did not differ significantly between VDS and control animals. None of the VDS animals had any other clinical signs, e.g. anorexia, and only a few of them were in poor condition, as measured by body condition score. We had only one case with a large amount of discharge. More severe causes of VDS, such as metritis, produce considerably larger amounts of discharge than the apparently less harmful causes (e.g. vaginitis) from a holistic point of view (Dee 1992). However, the VDS animals had lower odds of farrowing than control animals. Lameness, especially severe lameness, is a systemic condition resulting in a significant APR. Measurement of APPs may set up prognosis or aid in the follow-up of treatments for different conditions causing lameness.

## 9. CONCLUSIONS

1. PPV and lameness are common and VDS is rare in the Finnish loose-housed sow population.
2. VDS impaired fertility, whereas confinement to cages after mating and giving roughage to sows improved it. Lameness did not affect fertility.
3. High HI titres are more common in smaller herds and in older sows. None of the factors studied had an association with VDS. Animals housed on slatted floors had higher odds of being lame than animals housed on solid floors.
4. The agreement between ELISA and HI tests was moderate.
5. Vaginoscopy and bacteriology are valid tools in diagnosis of VDS. Susceptibility testing of antimicrobial treatments is indicated.
6. Lameness induced an acute-phase reaction, but VDS did not.

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## **12. ORIGINAL PAPERS**